28), wherein P is a proline residue, K is a lysine residue, I is an isoleucine residue, X is any amino acid residue, Q is a glutamine residue, T is a threonine residue, R is an arginine residue, and E is a glutamic acid residue. Examples of two preferred calcineurin antagonists are the peptides PKPKIIQTRRPE (SEQ ID No. 29) and PKPKINQTRRPG (SEQ ID No. 30).

On page 68, please replace the partial paragraph at the top of the page with:

reduction, is capable of transmembrane transport by receptor-mediated transcytosis and can therefor serve as an internalizing peptide for the subject transcellular peptides and peptidomimetics. Preferred growth factor-derived internalizing peptides include EGF (epidermal growth factor)-derived peptides, such as CMHIESLDSYTC (SEQ ID No. 31) and CMYIEALDKYAC (SEQ ID No. 32); TGF-beta (transforming growth factor beta)-derived peptides; peptides derived from PDGF (platelet-derived growth factor) or PDGF-2; peptides derived from IGF-I (insulin-like growth factor) or IGF-II; and FGF (fibroblast growth factor)-derived peptides.

On page 68, please replace the last full paragraph with:

A particularly preferred pH-dependent membrane-binding internalizing peptide in this regard is aa1-aa2-aa3-EAALA(EALA)4-EALEALAA-amide (SEQ ID No. 33), which represents a modification of the peptide sequence of Subbarao et al. (Biochemistry 26:2964, 1987). Within this peptide sequence, the first amino acid residue (aa1) is preferably a unique residue, such as cysteine or lysine, that facilitates chemical conjugation of the internalizing peptide to a targeting protein conjugate. Amino acid residues 2-3 may be selected to modulate the affinity of the internalizing peptide for different membranes. For instance, if both residues 2 and 3 are lys or arg, the internalizing peptide will have the capacity to bind to membranes or patches of lipids having a negative surface charge. If residues 2-3 are neutral amino acids, the internalizing peptide will insert into neutral membranes.

Please replace the partial paragraph at the bottom of page 69 with:

An exemplary accessory moiety in this regard would be a peptide substrate for N-myristoyltransferase, such as GNAAAARR (SEQ ID No. 34) (Eubanks et al., in: Peptides. Chemistry and Biology, Garland Marshall (ed.), ESCOM, Leiden, 1988, pp. 566-69) In this construct, an

a4

Please replace the last three paragraphs on page 71 with:

catatgggtggctgccgtggcgatatgttcggttgcggtgctcctccaaaaaagaagagaaaggtagctggattc (SEQ ID No. 35), which encodes the RGD/SV40 nucleotide sequence: MGGCRGDMFGCGAPPKKKRKVAGF (SEQ ID No. 36). In another embodiment, the protein can be engineered with the HIV-1 tat(1-72) polypeptide, e.g., as provided by the Nde1-EcoR1 fragment:catatggagccagtagatcctagactagagccctggaagcatccaggaagtcagcctaaaactgcttgtaccaattgctattgt aaaaagtgttgctttcattgccaagtttgtttcataacaaaagcccttggcatctcctatggcaggaagaagcggagacagcgacgaagacct cctcaaggcagtcagactcatcaagtttctctaagtaagcaaggattc (SEQ ID No. 37), which encodes the HIV-1 tat(1-72) peptide sequence: MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRRPP QGSQTHQVSLSKQ (SEQ ID No. 38). In still another embodiment, the fusion protein includes the HSV-1 VP22 polypeptide (Elliott G., O'Hare P (1997) Cell, 88:223-233) provided by the Nde1-EcoR1 fragment:

a5

Please replace the text on page 72 with:

cat atg acc tct cgc cgc tcc gtg aag tcg ggt ccg cgg gag gtt ccg cgc gat gag tac gag gat ctg tac tac acc ccg tct tca ggt atg gcg agt ccc gat agt ccg cct gac acc tcc cgc cgt ggc gcc cta cag aca cgc tcg cgc cag agg ggc gag gtc cgt ttc gtc cag tac gac gag tcg gat tat gcc ctc tac ggg ggc tcg tca tcc gaa gac gac gaa cac ccg gag gtc ccc cgg acg cgg cgt ccc gtt tcc ggg gcg gtt ttg tcc ggc ccg ggg cct gcg cgg gcg cct ccg cca ccc gct ggg tcc gga ggg gcc gga cgc aca ccc acc acc gcc ccc cgg gcc ccc cga acc cag cgg gtg gcg act aag gcc ccc gcg gcc ccg gcg gcg gag acc acc cgc ggc agg aaa tcg gcc

a6

cag cca gaa tcc gcc gca ctc cca gac gcc ccc gcg tcg acg gcg cca acc cga tcc aag aca ccc gcg cag ggg ctg gcc aga aag ctg cac ttt agc acc gcc ccc cca aac ccc gac gcg cca tgg acc ccc cgg gtg gcc ggc ttt aac aag cgc gtc ttc tgc gcc gcg gtc ggg cgc ctg gcg gcc atg cat gcc cgg atg gcg gcg gtc cag ctc tgg gac atg tcg cgt ccg cgc aca gac gaa gac ctc aac gaa ctc ctt ggc atc acc acc atc cgc gtg acg gtc tgc gag ggc aaa aac ctg ctt cag cgc gcc aac gag ttg gtg aat cca gac gtg gtg cag gac gtc gac gcg gcc acg gcg act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag gaa ttc (SEQ ID No. 39)

which encodes the HSV-1 VP22 peptide having the sequence:

MTSRRSVKSGPREVPRDEYEDLYYTPSSGMASPDSPPDTSRRGALQTRSRQRGEVRFVQ YDESDYALYGGSSSEDDEHPEVPRTRRPVSGAVLSGPGPARAPPPPAGSGGAGRTPTTAP RAPRTGRVATKAPAAPAAETTRGRKSAQPESAALPDAPASTAPTRSKTPAQGLARKLHF STAPPNPDAPWTPRVAGFNKRVFCAAVGRLAAMHARMAAVQLWDMSRPRTDEDLNE LLGITTIRVTVCEGKNLLQRANELVNPDVVQDVDAATATRGRSAASRPTERPRAPARSA SRPRRPVE (SEQ ID No. 40)

In still another embodiment, the fusion protein includes the C-terminal domain of the VP22 protein from, e.g., the nucleotide sequence (Nde1-EcoR1 fragment):

a6 cont

cat atg gac gtc gac gcg gcc acg gcg act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag gaa ttc (SEQ ID No. 41)

which encodes the VP22 (C-terminal domain) peptide sequence: MDVDAATATRGRSA-ASRPTERPRAPARSASRPRRPVE (SEQ ID No. 42)

In certain instances, it may also be desirable to include a nuclear localization signal as part of the RRPE peptide.

In the generation of fusion polypeptides including the subject RRPE peptides, it may be necessary to include unstructured linkers in order to ensure proper folding of the various peptide domains, and prevent steric or other interference of the heterologous domains with the

Please replace the last full paragraph on page 89 with:

a7 5' AGGAGGTGGATCTGC 3' (SEQ ID No. 43, corresponding to nucleotides 5-19 of the mouse Csp1 cDNA sequence shown in SEQ ID NO: 2)

Please replace the second full paragraph on page 127 with:

We then asked which of these Cspl mutants could interfere with calcium-induced NF-AT4 nuclear import in mammalian cells (Shibasaki et al., 1996). As might be expected, none of the Csp1 mutants that failed to bind calcineurin *in vitro* in inhibited calcineurin-dependent NF-AT4 nuclear import in BHK cells (Fig. 5). Interestingly, truncation mutants containing either of the calcineurin-binding domains of the C-terminal half of Csp1 were effective inhibitors of NF-AT4 nuclear import, suggesting that these mutants interfered with substrate recognition, phosphatase activity, or both. One sequence element (ERMRRP, SEQ ID No. 44) in the distal portion of the C-terminal half of Cspl appeared similar to the consensus autoinhibitory domain of mammalian calcineurin A (ERMPPRRD, SEQ ID No. 45; Hashimoto et al., 1990). Csp2 lacks the ERM sequence, but shares considerable homology with Cspl in an adjacent sequence block that is highly conserved in the Csps and contains basic residues (PKPKIIQTRRPE, SEQ ID No. 29) (Fig. **20**). Separate mutations affecting the ERM, RRPE, or other conserved sequence elements such as LIS108, did not prevent Cspl's inhibition of calcineurin-dependent translocation of NF-AT to the nucleus, nor Cspl binding to calcineurin in vitro. However, when these Csp1 mutants were assessed for their ability to block hydrolysis of pNPP by calcineurin, the one lacking the RRPE sequence proved remarkably defective in this assay (Fig. **20**). Together, these data suggest that the calcipressins inhibit calcineurin by duel mechanisms involving competition for substrate binding as well as suppression of catalytic activity via the RRPE "pseudosubstrate" domain.

a8

Please replace any sequence listing currently in the application with the hard copy of the sequence listing included herewith.

The replacement paragraphs presented above incorporate changes as indicated by the marked-up versions below.

In certain embodiments, the calcineurin antagonists of the invention comprise the polypeptide sequence RR. In preferred embodiments, the calcineurin antagonists of the invention comprise a polypeptide sequence RRP; or, more preferrably, the sequence RRPZ, wherein Z is any amino acid residue other than a serine or a threonine; or, still most preferrably, RRPY, wherein Y is an alanine residue, a glycine residue or a glutamic acid residue. In still more preferred embodiments, the calcineurin antagonists comprise an RRPE motif; or, most preferrably, a sequence motif conforming to the general structure PKPKIXQTRRPE (SEQ ID No. 28), wherein P is a proline residue, K is a lysine residue, I is an isoleucine residue, X is any amino acid residue, Q is a glutamine residue, T is a threonine residue, R is an arginine residue, and E is a glutamic acid residue. Examples of two preferred calcineurin antagonists are the peptides PKPKIIQTRRPE (SEQ ID No. 29) and PKPKINQTRRPG (SEQ ID No. 30).

reduction, is capable of transmembrane transport by receptor-mediated transcytosis and can therefor serve as an internalizing peptide for the subject transcellular peptides and peptidomimetics. Preferred growth factor-derived internalizing peptides include EGF (epidermal growth factor)-derived peptides, such as CMHIESLDSYTC (SEQ ID No. 31) and CMYIEALDKYAC (SEQ ID No. 32); TGF-beta (transforming growth factor beta)-derived peptides; peptides derived from PDGF (platelet-derived growth factor) or PDGF-2; peptides derived from IGF-I (insulin-like growth factor) or IGF-II; and FGF (fibroblast growth factor)-derived peptides.

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this peptide sequence, the first amino acid residue (aa1) is preferably a unique residue, such as cysteine or lysine, that facilitates chemical conjugation of the internalizing peptide to a targeting protein conjugate. Amino acid residues 2-3 may be selected to modulate the affinity of the internalizing peptide for different membranes. For instance, if both residues 2 and 3 are lys or arg, the internalizing peptide will have the capacity to bind to membranes or patches of lipids having a negative surface charge. If residues 2-3 are neutral amino acids, the internalizing peptide will insert into neutral membranes.

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catatgggtggctgccgtggcgatatgttcggttgcggtgctcctccaaaaaagaagagaaaggtagctggattc (SEQ ID No. 35), which encodes the RGD/SV40 nucleotide sequence: MGGCRGDMFGCGAPPKKKRKVAGF (SEQ ID No. 36). In another embodiment, the protein can be engineered with the HIV-1 tat(1-72) polypeptide, e.g., as provided by the Nde1-EcoR1 fragment:catatggagccagtagatcctagactagagccctggaagcatccaggaagtcagcctaaaactgcttgtaccaattgctattgt aaaaagtgttgctttcattgccaagtttgtttcataacaaaagcccttggcatctcctatggcaggaagaagcggagacagcgacgaagacct cctcaaggcagtcagactcatcaagtttctctaagtaagcaaggattc (SEQ ID No. 37), which encodes the HIV-1 tat(1-72) peptide sequence: MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRRPP QGSQTHQVSLSKQ (SEQ ID No. 38). In still another embodiment, the fusion protein includes the HSV-1 VP22 polypeptide (Elliott G., O'Hare P (1997) Cell, 88:223-233) provided by the Nde1-EcoR1 fragment:

cat atg acc tct cgc cgc tcc gtg aag tcg ggt ccg cgg gag gtt ccg cgc gat gag tac gag gat ctg tac tac acc ccg tct tca ggt atg gcg agt ccc gat agt ccg cct gac acc tcc cgc cgt ggc gcc cta cag aca cgc tcg

cgc cag agg ggc gag gtc cgt ttc gtc cag tac gac gag tcg gat tat gcc ctc tac ggg ggc tcg tca tcc gaa gac gac gaa cac ccg gag gtc ccc cgg acg cgg cgt ccc gtt tcc ggg gcg gtt ttg tcc ggc ccg ggg cct gcg cgg gcg cct ccg cca ccc gct ggg tcc gga ggg gcc gga cgc aca ccc acc acc gcc ccc cgg gcc ccc cga acc cag cgg gtg gcg act aag gcc ccc gcg gcc ccg gcg gcg gag acc acc cgc ggc agg aaa tcg gcc cag cca gaa tcc gcc gca ctc cca gac gcc ccc gcg tcg acg gcg cca acc cga tcc aag aca ccc gcg cag ggg ctg gcc aga aag ctg cac ttt agc acc gcc ccc cca aac ccc gac gcg cca tgg acc ccc cgg gtg gcc ggc ttt aac aag cgc gtc ttc tgc gcc gcg gtc ggg cgc ctg gcg gcc atg cat gcc cgg atg gcg gcg gtc cag ctc tgg gac atg tcg cgt ccg cgc aca gac gaa gac ctc aac gaa ctc ctt ggc atc acc acc atc cgc gtg acg gtc tgc gag ggc aaa aac ctg ctt cag cgc gcc aac gag ttg gtg aat cca gac gtg gtg cag gac gtc gac gcg gcc acg gcg act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag gaa ttc (SEQ ID No. 39)

which encodes the HSV-1 VP22 peptide having the sequence:

MTSRRSVKSGPREVPRDEYEDLYYTPSSGMASPDSPPDTSRRGALQTRSRQRGEVRFVQ
YDESDYALYGGSSSEDDEHPEVPRTRRPVSGAVLSGPGPARAPPPPAGSGGAGRTPTTAP
RAPRTGRVATKAPAAPAAETTRGRKSAQPESAALPDAPASTAPTRSKTPAQGLARKLHF
STAPPNPDAPWTPRVAGFNKRVFCAAVGRLAAMHARMAAVQLWDMSRPRTDEDLNE
LLGITTIRVTVCEGKNLLQRANELVNPDVVQDVDAATATRGRSAASRPTERPRAPARSA
SRPRRPVE (SEQ ID No. 40)

In still another embodiment, the fusion protein includes the C-terminal domain of the VP22 protein from, e.g., the nucleotide sequence (Nde1-EcoR1 fragment):

cat atg gac gtc gac gcg gcc acg gcg act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag gaa ttc (SEQ ID No. 41)

which encodes the VP22 (C-terminal domain) peptide sequence: MDVDAATATRGRSA-ASRPTERPRAPARSASRPRRPVE (SEQ ID No. 42)

In certain instances, it may also be desirable to include a nuclear localization signal as part of the RRPE peptide.

In the generation of fusion polypeptides including the subject RRPE peptides, it may be necessary to include unstructured linkers in order to ensure proper folding of the various peptide domains, and prevent steric or other interference of the heterologous domains with the

5' AGGAGGTGGATCTGC 3' (SEQ ID No. 43, corresponding to nucleotides 5-19 of the mouse Csp1 cDNA sequence shown in SEQ ID NO: 2)

We then asked which of these Cspl mutants could interfere with calcium-induced NF-AT4 nuclear import in mammalian cells (Shibasaki et al., 1996). As might be expected, none of the Csp1 mutants that failed to bind calcineurin *in vitro* in inhibited calcineurin-dependent NF-AT4 nuclear import in BHK cells (Fig. 5). Interestingly, truncation mutants containing either of the calcineurin-binding domains of the C-terminal half of Csp1 were effective inhibitors of NF-AT4 nuclear import, suggesting that these mutants interfered with substrate recognition, phosphatase activity, or both. One sequence element (ERMRRP, SEQ ID No. 44) in the distal portion of the C-terminal half of Cspl appeared similar to the consensus autoinhibitory domain of mammalian calcineurin A (ERMPPRRD, SEQ ID No. 45; Hashimoto et al., 1990). Csp2 lacks the ERM sequence, but shares considerable homology with Cspl in an adjacent sequence block that is highly conserved in the Csps and contains basic residues (PKPKIIQTRRPE, SEQ ID No. 29) (Fig. **20**). Separate mutations affecting the ERM, RRPE, or other conserved sequence elements such as LIS108, did not prevent Cspl's inhibition of calcineurin-dependent translocation of NF-AT to the nucleus, nor Cspl binding to calcineurin in vitro. However, when these Csp1 mutants were assessed for their ability to block hydrolysis of pNPP by calcineurin, the one lacking the RRPE sequence proved remarkably defective in this assay (Fig. **20**). Together, these data suggest that the calcipressins inhibit calcineurin by duel mechanisms involving competition for substrate binding as well as suppression of catalytic activity via the RRPE "pseudosubstrate" domain.